DATA EVALUATION RECORD

Flumethrin
PC Code: 036007
TXR#: 0055615
MRID#: 48240232

Developmental Neurotoxicity Study - Rats
OPPTS 870.6300
OECD 426

A Developmental Neurotoxicity Study with Technical Grade Flumethrin in Wistar Rats

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study – Rat (OPPTS 870.6300; OECD 426).

PC CODE: 036007 **DP BARCODE:** D385868
TXR #: 0055615

TEST MATERIAL: Flumethrin,
Cyano (4-fluoro-3-phenoxyphenyl)methyl 3-[2-chloro-2-(4-chlorophenyl)ethenyl]-2-dimethylcyclopropanecarboxylate.

SYNONYMS: Bayticol; Bayticol Pour-on; Bayvarol; FCR 2769; trans-3-(2-Chloro-2-(4-chlorophenyl)ethenyl)-2,2-dimethyl-cyclopropanecarboxylic acid, cyano(4-fluoro-3-phenoxyphenyl)methyl ester; alpha-Cyano-4-fluoro-3-phenoxybenzyl 3-(2-chloro-2-(4-chlorophenyl)vinyl)-2,2-dimethylcyclopropanecarboxylate

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SPONSOR: Bayer Health Care AG, Alfred Nobel Str. 50, 40789 Monheim, Germany.

EXECUTIVE SUMMARY

In a developmental neurotoxicity study (MRID # 48240232), Technical grade Flumethrin (purity 96.0%, Batch no. KP03HHE04) was administered once daily by gavage at nominal doses of 0, 0.5, 1 or 2 mg/kg/day to mated female Wistar rats (30 females/dose) from gestation day (GD) 6 through lactation day (LD) 21. Analytically confirmed doses were 0, 0.51, 0.97 and 1.90 mg/kg/day for females.

Concentration in the vehicle, as well as the homogeneity and stability, was confirmed. On postnatal day (PND) 4, litters with a minimum of seven pups, including at least three per sex, were culled to yield, as closely as possible, four males and four females. Subsets of surviving offspring, representing at least 17-20 litters per dose level, were subjected to evaluation using the following observations and measurements: detailed clinical observations and a functional observational battery, surface righting, preputial separation or vaginal patency, body weight, automated measures of activity (figure-eight maze), auditory startle habituation, learning and memory (passive avoidance after weaning and a water maze task beginning on PND 60 ±2 days) and an ophthalmic examination. Neural tissues were collected from 10/sex/dose level (representing 20 litters) on PND 21 (brain only) and at study termination (approximately 75 days of age) for microscopic examination and morphometry.

Maternal Results:

There were no treatment-related findings at dose levels of 0.51, 0.97 and 1.90 mg/kg bw/day during gestation or lactation. However, the only compound-related effect was evident in food consumption at the high dose 1.90 mg/kg bw/day during gestation. During GD 13-20, the high-dose (1.90 mg/kg

bw/day) females consumed an average of 15 % less feed (g/animal/day) than controls. This difference in food consumption for high-dose females was associated with an average 9 % decrease in weight gain from GD 0 to 20 and a slightly lower (3 %) body weight for high-dose females, compared to controls, on GD 20.

The maternal **LOAEL is 2.0 mg/kg bw/day** and **NOAEL is 1.0 mg/kg bw/day**.

Offspring Results:

There were no treatment-related findings at any dose levels except for effects on body weight and body weight gain at the high dose (1.90 mg/kg/day).

Body weight was statistically decreased on PND 11 in high-dose males (-8%) and females (-6%). On PND 17, the difference in body weight was statistically significant in males (-8%) but not in females (-5%). In addition, body weight remained on average 6% less than controls for high-dose males on PND 21, which was not statistically significant, and recovered to within 4% less than controls for high-dose females. Low-dose females had a significantly lower (-6%) body weight on PND 11, which was not attributed to treatment since this was the only time point where body weight was considered to be different from control and there was no similar difference in females at the mid-dose nor in males at the low- or mid-dose levels.

Body weight gain was statistically decreased for various measurement intervals in high-dose animals. For PND 4-11, weight gain was statistically decreased in high-dose (1.90 mg/kg/day) males (9 %) and high-dose males and females combined (9 %), with an 8 % lower weight gain for high-dose females. Body weight gain was similarly statistically decreased on PND 4-17 in high-dose males (9 %) and high-dose males and females combined (7%) and in high-dose males only on PND 11- 17 (8%). There were no statistical differences from control in low- or mid-dose males or females at any time.

After weaning and discontinuation of treatment, the difference in body weight for high-dose males that was evident prior to weaning persisted to study termination (an average 6-7 % less than controls). For females, there was no difference from controls after weaning at any dietary level.

Thus, the offspring **LOAEL is 2 mg/kg bw/day**, based on decreased body weight and body weight gain in males and females. The offspring **NOAEL is 1 mg/kg bw/day**.

Based on maternal and offspring results, it is concluded that technical grade flumethrin, when administered at the highest dose of 2 mg/kg/day to pregnant rats from GD 6 through LD 21, is not a developmental neurotoxicant. The only compound-related effects occurred at the highest dose level and consisted of decreases in body weight and body weight gain in maternal females and offspring (males and females), and in food consumption (in dams only).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (870.6300 and OECD 426) for a developmental neurotoxicity study in rats.

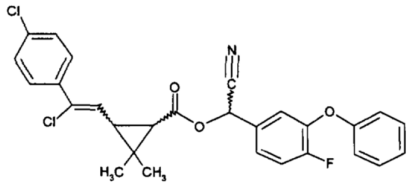
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** Flumethrin

Description:	Brown liquid or solid melt
Lot/Batch#:	KP03HHE04
Purity:	96.0%
CAS #:	69770-45-2
Compound Stability:	Stable at room temperature (20±5ºC)
Structure:	



2. **Vehicle:** Corn oil (Bayer batch no. 15-05051).

3. **Test animals**

Species:	Rat,	
Strain:	Wistar HAN CRL:WI (HAN)	
Age/ weight at study initiation:	Approximately 15 weeks for males; 12 weeks for females Females: 167.6 – 226.1 g Males have no specified weight requirements	
Source:	Charles River Laboratories, Inc., (Raleigh, NC)	
Housing	Individually housed in suspended stainless steel wire-mesh cages, except with one male each during cohabitation, with sanitized cage board in the bedding tray. Individually in plastic cages with corn cob bedding during gestation and lactation.	
Diet:	Purina Mills Rodent Lab Chow 5002 in meal form provided for *ad libitum*	
Water:	Tap water (Kansas City Missouri Municipal Water) was provided *ad libitum*.	
Environmental conditions:	**Temperature:**	18-26 ˚C
	Humidity:	30 – 70%
	Air changes:	10-air changes per hour
	Photoperiod:	12 hr light/ 12 hr dark
Acclimation period:	6 days minimum under test conditions with an evaluation of the health status	

B. STUDY DESIGN AND PROCEDURES:

1. **In-Life Dates**:

Study starting date:	May 8, 2006
Study termination date:	August 18, 2006

2. **Study Schedule:**

The maternal animals (P-generation) were mated and assigned to study as they were determined to be sperm positive. The test substance was administered by gavage to the dams from gestation day 6 through day 21 of lactation / postnatal development. Pups were weaned on postnatal day

(PND) 21, after which time maternal animals were sacrificed. The offspring (Fl generation) that remained on study after weaning were sacrificed at study termination on PND 75 (±5 days).

3. Mating Procedure:

Mating was managed by co-housing one female with one male, for a maximum of five consecutive days. Each female was examined each morning for a vaginal plug and vaginal smears were taken and examined for the presence of sperm. The day on which insemination was observed in the vaginal smear was designated day 0 of gestation (GD 0) for that female. After successful mating, each pregnant female was housed individually in a plastic nesting cage, where it was maintained through gestation and lactation. Typically, females that are not sperm positive are sacrificed without a necropsy examination.

4. Animal Assignment:

Mated female were assigned randomly using computer-based application (SAS, Version 6.09E) to dose groups as indicated in Table 1. The body weights of parental (P)-generation females were within 20 % of the mean weight as required by the guidelines. Animals were assigned an identification number that specified the sex, dose level and cage number, and identified it with the study. P-generation males served only as breeders. As such, they had no specific weight requirements and were arbitrarily selected for co-housing with females.

Parental (P)-generation males and females were identified by cage card and tail mark (males) or tail tattoo (females). Fl-generation animals that were born alive were identified by tattoo; pups that were found dead were identified with a marking pen.

Offspring were assigned to testing subgroups at the time of litter standardization on postnatal day 4 (Table 1). An animal allocation program written in SAS [1] was used to assign offspring to the following four sets (designated A-D) for assessment at each age. One male and/or female per litter (approximately 16 (minimum 10)/sex/dose level, representing at least 20 litters per level): Motor activity (Set A), Auditory Startle (Set B), Passive Avoidance, Water Maze and Functional Observational Battery (Set C). On PND21, the whole brain was collected from a separate group of randomly selected offspring (Set D; 10/sex/dose level; representing 20 litters per level) for micropathological examination and morphometric analysis. The remaining pups assigned to Set D (~10/sex/dose level) were reserved for possible use as replacement animals or were otherwise sacrificed on PND21 without necropsy examination.

At approximately 50-60 days of age, randomly selected animals (a minimum of 10/sex/dose level, representing at least 20 litters per level) from Sets A, B and C were subjected to an ophthalmologic examination. At termination (PND75 (± 5 days)), these animals were anesthetized and sacrificed by perfusion, with neural and muscle tissues collected for microscopic examination. At termination on PND75 (±5 days), brains were collected from additional randomly selected animals (10/sex/dose group; representing 20 litters per level). These brains were weighed (fresh tissue weight) and then discarded.

The remaining animals assigned to sets A-C were sacrificed without routine gross necropsy examination or collection of tissues.

TABLE 1. Study Design

Experimental parameter	Dose Level (mg/kg bw/day)			
	Control	0.5	1.0	2.0
Maternal Animals				
No. of maternal animals assigned	30	30	30	30

FOB (GD 13, 20 / LD 11, 21)	30/10	30/10	30/10	30/10
Offspring				
Detailed clinical observations / FOB PND 4, 11, 21, 35 (±1), 45 (±1), 60 (±2)	20 (min.10)/sex	19-20 (min.10)/sex	19-20 (min 10)/sex	19-20 (min. 10)/sex
Motor activity PND 13, 17, 21, 60 (±2)	20 (min. 10)/sex	20 (min. 10)/sex	20 (min. 10)/sex	18-20 (min. 10)/sex
Auditory startle habituation PND 23, 60 (±2)	18-20 (min. 10)/sex	19-20 (min. 10)/sex	18-20 (min. 10)/sex	18-20 (min. 10)/sex
Learning and memory PND 23, 30, 60 (±2), 67 (±2)	16 (min. 10)/sex	16 (min. 10)/sex	15-16 (min. 10)/sex	15-16 (min. 10)/sex
Brain weight PND 21 PND 75 (±5)	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex
Neuropathology PND 21 PND 75 (±5)	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex

The method of animal assignment minimized potential problems related to litter effects, by using at least one pup/litter. For FOB and motor activity testing, the same individual animals were evaluated at all scheduled time points. For the selection of animals and testing paradigms for cognitive (learning and memory) assessment, the same animals were used for assessments at the weanling and adult ages, but different tests were used at the two ages.

5. Test Selection Rational:

The rationale for dose selection is based, in part, on the results of a two-generation reproduction study in Wistar rats that were exposed to technical-grade Flumethrin at dietary levels of 0, 1, 5 or 50 ppm (Dotti et. al., 1992). In that study, the 50 ppm (2.5-4.9 mg/kg/day for the P-generation) dietary level produced overt toxicity in the P-generation females, including clinical signs (skin lesions) and decreased food consumption. Compound-related effects in the Fl-generation at 50 ppm included fetuses with various alterations (e.g., cramped or bent posture and stiff limbs in caudal position), increased breeding loss, and decreased survival and decreased body weight gain. Around PND 14, all high-dose pups were also reduced in size and were cool-to-touch. There were no compound-related effects in any generation at the 5 ppm dietary level (approximately 0.51 mg/kg/day).

The results from a developmental toxicity study in Wistar rats were also considered in dose selection (Klaus, 1999). In that study, inseminated females received Flumethrin by gavage in corn oil at nominal doses of 0, 0.75, 2 or 5 mg/kg/day on GD 6-19, with fetuses delivered by Cesarean section on GD 20. Effects in inseminated females at 2 mg/kg/day (slight) and 5 mg/kg/day included pruritus (scratching), salivation, reduced feed consumption and body weight gain. Reproductive parameters were not affected by Flumethrin at any dose level. However, fetal toxicity was evident at 5 mg/kg/day, including increased incidence of necrotic placental borders, reduced placental weight, and distinctly reduced fetal weight, as well as slightly increased incidences of common malformations. Such findings were not evident at lower dose levels.

Finally, a pilot study was conducted to assess toxicity and determine whether the offspring are exposed via the transfer of Flumethrin through the milk during lactation (Sheets, 2006). In this study, pregnant female Wistar rats were treated daily by gavage to a nominal concentration of 0, 2 or 4 mg/kg/day Flumethrin (in corn oil, 5 ml/kg) from GD 6 through lactation day 21, to provide a minimum 10 suitable litters at each age. The offspring from each litter were sacrificed on lactation day 4 (pooled culls) or on lactation day 11, 17 or 21 (one male or female/litter at each age; 5/sex/age, representing 10 litters at each age) to measure the concentration of Flumethrin in the whole pup. The 4 mg/kg/day dose level was terminated on GD 16 due to excessive toxicity, including clinical signs (hunched posture, ataxia, decreased activity,

salivation, lacrimation, staining (lacrimal, nasal, perianal and oral)) and a marked decrease in body weight (13 % lower on GD 13) and food consumption (58 % lower on GD 6-13) during pregnancy, with one animal euthanized in a severe condition on GD 10. At 2 mg/kg/day, compound-related effects were evident in the dams (i.e., reduced body weight during gestation and lactation and reduced body weight gain and food consumption during gestation, lacrimation (during gestation), nasal stain (during gestation and lactation), salivation (during gestation and lactation) and oral stain (during gestation and lactation). Salivation and oral stain observed in dams during gestation and lactation were considered to be secondary to paresthesias resulting from direct contact of the test substance with the oral mucosa and were not systemic effects. Signs of toxicity in the 2 mg/kg/day offspring included a 10 % lower birth weight in males and females combined. In addition, pup body weights at 2 mg/kg/day (males and females combined) averaged 9-14 % less than control on PND 4 through PND 21. The analysis of tissue (i.e., whole body) from PND 11 pups verified the presence of Flumethrin in all 10 animals (average 63 ppb), which verified that the pups were exposed during lactation. Based on this result, the tissues were not assayed at other ages.

Based on these combined results, the dose levels selected for this developmental neurotoxicity study were 0, 0.5, 1 and 2 mg/kg/day. The 2 mg/kg/day dose was selected as a maximum dose the animals will tolerate without excessive toxicity. The 1 mg/kg/day dose was selected as an intermediate dose that may produce slight effects and 0.5 mg/kg/day was not expected to produce any compound-related effects and therefore was selected to establish an overall NOAEL.

6. Dosage Administration:

The dams were treated once daily by gavage beginning on GD 6 and continuing through lactation day 21, except on GD 21 (the anticipated day of parturition). Animals were not fasted overnight before treatment.

7. Dosage Preparation and Analysis:

Dose formulations were prepared weekly by heating the test substance to 70° C in a water bath. Appropriate amounts of the test substance were then mixed in corn oil at a dosing volume of 5 ml/kg. Dose formulations were vortexed and stirred continuously during use and were stored at room temperature. The stability at room temperature (~ 22°C) and homogeneity of the test substance in corn oil were established by analysis of samples at nominal concentrations of 0.04, 1.0 and 3.0 mg/ml. Homogeneity was accepted if the percent relative standard deviation (%RSD) was < 5 %. The concentration of the test substance in the vehicle was measured for doses that were used for all weeks of the study. The concentration of Flumethrin in the vehicle was measured by high-performance liquid chromatographic/ultra violet (HPLC/UV) analysis (Moore and Neal, 2007).

Homogeneity analysis: Homogeneity of the test substance in the vehicle was accepted for the range of concentrations that bracket those used in the present study. These concentrations of 0.04, 1 and 3 mg/ml (equivalent to doses of 0.2, 5 and 15 mg/kg) had percent relative standard deviations (%RSD) of 1.9 %, 1.1 % and 1.2 %, respectively.

Stability analysis: The stability of Flumethrin in the vehicle (corn oil) was established at room temperature for nominal concentrations of 0.04, 1 or 3 mg/ml, with no appreciable decrease in concentration with ten days of storage.

Concentration analysis: Actual (analytically-determined) concentrations of the active ingredient in the nominal 0.5, 1and 2 mg/kg/day dose levels used in this study averaged

102 %, 97 % and 95 % of the nominal concentrations. Based on these results, the mean analytically confirmed dosages for this study were 0, 0.51, 0.97 and 1.9 mg/kg/day for males and females.

C. OBSERVATIONS / METHODS

1. In-Life Observations

A. Maternal Animals

1) **Clinical Observation:** Cage-side observations were performed at least once daily for mortality and clinical signs of moribundity. Detailed physical examinations for clinical signs of toxicity were carried out and recorded once each week.

2) **Detailed Observations:** A detailed evaluation of the dams for clinical signs with a physical examination was conducted once daily from the initiation of exposure (GD 6) through lactation day 21.

3) **Functional Observational Battery:** Animals that were presumed to be pregnant (approximately 30 per dose level) were observed on GD 13 and GD 20 and a minimum 10 dams/dose level that were maintained on study with suitable litters were also observed on LD 11and LD21. All observations were performed by an individual who was unaware of each animal's dose group assignment. This observational battery included, but was not limited to, assessments (with severity scoring) of lacrimation, salivation, piloerection, exophthalmia, urination, defecation, pupillary function, palpebral closure, convulsions, tremor, abnormal movements, unusual behaviors, posture and gait abnormalities.

4) **Body Weight and Food Consumption:** Body weight and food consumption were measured once weekly during gestation and lactation, as follows: Gestation days 6-13, 13-20 and lactation days 0-7, 7-14 and 14-21. In addition, dams were weighed on GD 0 and LD 4. Measures of food consumption may have included consumption by the pups, especially during the third week of lactation. Fresh feed and clean feeders were provided weekly.

5) **Delivery and Culling:** Each dam was evaluated daily for evidence of delivery from GD 20 to the completion of delivery, which was designated lactation day 0 (LD 0) for the dam and postnatal day 0 (PND 0) for the pups. Litter size (the number of pups delivered) and pup "status" at birth were recorded for each litter. If a dam delivered fewer than three pups per sex or if the litter size decreased to fewer than seven pups by PND 4, the dam and litter were sacrificed without necropsy examination. For litters that met the minimum size requirements, the size of each litter was adjusted on PND 4 to yield, as closely as possible, four males and four females. Adjustments of litters were made by random selection of the pups using SAS applications. If the number of male or female pups was less than four, a partial adjustment was made (e.g., three females and five males). If there were more than 23 acceptable litters for any dose level, the surplus litters were sacrificed on PND 4 after weighing without routine necropsy, with preference given to retaining litters with a full complement of four males and four females. Culled dams and pups were sacrificed by CO_2 asphyxiation and decapitation, respectively. Dams with insufficient litters were also sacrificed by carbon dioxide (CO_2) asphyxiation.

6) **Moribund Animals and Animals Found Dead:** Parental-generation males and females that were found moribund (if any) while on study were sacrificed by CO_2 asphyxiation. Dams that were found dead or moribund (if any) underwent a gross necropsy examination, with possible collection of tissues, if the study director determined this was necessary to assist in determining

the cause of death. P-generation males that were found dead or moribund (if any) did not undergo a necropsy examination and were disposed of without routine collection of tissues.

7) **Termination:** P-generation males and females were sacrificed by CO_2 asphyxiation. A gross necropsy examination was not performed on P-generation males or females.

Males: Following co-habitation, males were sacrificed by CO_2 asphyxiation and discarded unless an alternative use was found.

Females: Dams were sacrificed on LD 21, following the weaning of their respective litters. Females that were sperm positive and/or had an internal vaginal plug, but did not deliver, were generally sacrificed on GD 24 without necropsy examination.

B Offspring:

1) **Litter Observations**: The day of completion of parturition was designated as lactation day (postnatal day) 0. Following parturition, pups were examined for ano-genital distance to establish their gender, and then were tattooed and weighed. Live pups were counted, sexed and weighed individually for each litter on postnatal days 0, 4, 11,17, and 21. Offspring were examined cage-side for gross signs of mortality or morbidity daily throughout lactation. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration. More detailed observations for clinical signs were made once daily before weaning and once weekly thereafter. These observations were performed by an individual who was aware of assignments to dose level.

After weaning on PND 21, the remaining pups were weighed once weekly, as well as when vaginal patency or balanopreputial separation was first evident, with detailed observations for clinical signs performed once weekly. Food consumption was not measured after weaning. Fresh feed and clean feeders were provided every two weeks.

2) **Developmental Landmark:** Beginning on PND 38, male offspring were examined daily for balanopreputial separation. On PND 29, female offspring were examined daily for vaginal patency. Selected males and females were examined daily on PND 4 for surface righting. On PND 21, all pups were also tested for the presence of pupil constriction.

3) **Postweaning Observations:** After weaning on PND 21, offspring were examined twice daily for mortality, and cage-side observations were conducted once daily. Individual offspring body weight data were recorded weekly, as well as on the day that vaginal patency or balanopreputial separation was achieved.

4) **Body Weight and Food Consumption:** Surviving pups were weighed on PND 0, 4, 11, 17, and 21, and once weekly thereafter. The individual pups were also weighed when vaginal patency or balanopreputial separation were first evident. Food consumption was not measured after weaning on PND 21. Fresh feed and clean feeders were provided every two weeks.

5) **Neurobehavioral Evaluations:**

i) **Functional Observational Battery (FOB):** On postnatal days 4, 11, 21, 35 (± 1 day), 45 (± 1 day), and 60 (±2 days), approximately 20 offspring/sex/group (representing at least 20 litters per level) were examined outside the home cage in an FOB assessment, as appropriate for the developmental stage involved. This evaluation was performed according to the procedures described for maternal animals (see above), using standardized procedures. The only difference is that the neonates (i.e., PND 4 and 11) were not evaluated in the open field, since this is

routinely done only if the observer considers it necessary for evaluation and this was not the case in the present study.

ii) Motor Activity Testing: Motor activity was measured for approximately 20 rats/sex/dose (representing at least 20 litters per level) on PND 13, 17, 21 and 60 (±2 days). The same offspring were evaluated in the figure-eight maze for 60 minutes at each time point, using a computer-automated system (Universal Maze Monitoring System, Version 1.41, Columbus Instruments, Columbus, OH) and personal computer for automated data collection. Broad spectrum background noise [74+2dB(A)] was provided throughout the test to minimize acoustical variations during testing. The uniformity of light intensity (100+70 Lux) over each maze was verified daily. Motor and locomotor activities were examined as total activity counts (beam interruptions) for the 60-minute session and as activity during each ten-minute interval. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity over consecutive intervals of the test session.

iii) Auditory Startle Reflex Habituation: Auditory startle reflex habituation testing was performed in approximately 20 rats/sex/dose (representing at least 20 litters per level) on postnatal days 23 and 60 (+2 days), using an automated system. A personal computer was used to control the operation of an integrated startle response test system (Coulbourn Acoustic Startle, Version 3.210-00, Coulboum Instruments, Allentown, PA) and for automated data collection. Groups of four animals (maximum) were tested simultaneously within each of two startle system enclosures. The test session consisted of 50 trials that began following approximately a 5-minute adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus at 10-sec intervals. The average response amplitude and the magnitude of decrease (habituation) over blocks of ten trials were compared among the dosage groups. Data collection began with the presentation of S2 and continued thereafter for 200 msec. The analog signal for each response output (measured in mV) was digitized at one kHz (i.e., one sample/msec for 200 msec) and converted to grams using a previously determined calibration curve for each load cell. Peak response amplitude (g) and latency (msec) measurements were taken from each animal's individual response curve. Baseline was defined as the average force (g) exerted on the platform during the first 8 msec following the onset of S2, a time period that precedes response onset. This baseline value was taken to represent an approximate body weight measurement that was used to verify that the equipment used to measure the response amplitude was functioning properly. Response amplitude is defined as the maximum value of the average curve, minus the baseline (i.e., removing the animal's body weight from the measurement). Latency to peak is the time (msec) following the onset of S2 when the peak response amplitude occurs.

iv) Learning and memory testing: Learning and memory testing was performed in approximately 16 rats/sex/dose (minimum 10 offspring/sex/dose). The same set of animals was used for testing passive avoidance (on PND 23 and 30) and water maze (PND 60 (±2 days) and again seven days later).

Postweaning - Passive avoidance: Animals were tested for acquisition on PND 23 and for retention on PND 30. Testing was conducted using equipment and computer programs from Coulbourn Instruments (Graphic State Notation 2 Version 2.002-00, Allentown, PA). A personal computer was used to control the operation of the equipment and for automated data collection. Testing took place in individual isolation cubicles, each housing a single shuttle cage. Each isolation cubicle was lined with foam insulation to attenuate sound in the chamber and had a fan with a baffled air intake and exhaust system for ventilation. The shuttle cage consisted of a Plexiglas and stainless-steel rectangular chamber fitted with front-loading access.

Each shuttle cage (15 inches wide x 7.25 inches deep) was separated into two compartments of equal size (approximately 7x7 inches) by a wall that supported a centrally-located sliding (guillotine-type) door. The two compartments were identical, except that the walls in one compartment were lined with black film (dark-side) and the walls in the other compartment were not lined and it was illuminated during the test with a high-intensity lamp. The lamp was switched on to illuminate the light compartment at the start of each trial and remained on until either the animal crossed to the dark compartment or the trial ended. The floor of the cage consisted of a grid of stainless-steel bars. The movement of the animal from the starling (light) side to the dark compartment was detected by a photocell system. A Coulbourn solid-state scanning shock generator was used to deliver a brief (0.5 sec) pulse of mild (0.5 mA) distributed shock to the grid floor when the animal crossed to the dark compartment.

After adaptation, individual animals were placed individually into the "lighted" compartment of a conditioning apparatus (the shuttle cage), facing toward the light. After approximately 60 seconds, the trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that each rat was provided access to the non-illuminated side of the cage. When the rat crossed into the dark compartment, the door automatically closed, the shock was delivered, and the light switched off - signaling the end of that trial. At that time, the animal was returned promptly to the holding cage to wait for the next trial. If the rat failed to cross within 180 sec, it was returned to the holding cage and the latency assigned an arbitrary score of 180. The procedure was repeated until either the rat remained in the lighted compartment for 180 sec on two consecutive trials or until 15 trials had elapsed, whichever occurred first. Rats that failed to meet the criterion during the learning phase were assigned a value of 15 for the trials-to-criterion variable. The test was repeated one week later. For this second trial, rats were placed in the illuminated side of the apparatus, given a 20-sec acclimation period, and the latency to enter the dark side recorded.

Animals that either failed to reach criterion performance within 15 trials or failed to cross during the first two trials during acquisition were excluded from the retention phase of the experiment. The dependent measures were the number of trials-to-criterion, latency to cross on Trial 1 and Trial 2 (learning phase only) and the number of rats/group that failed to reach criterion within 15 trials (learning phase only).

Adult (PND 60) Offspring - Water maze: Animals were tested on PND 60 (±2 days), and again seven days later. Only animals that demonstrated acquisition were tested for retention. The water in the M-maze was maintained at 22 +1 °C. The mazes were constructed of opaque Plexiglas, with corridors approximately five inches wide and walls approximately 16 inches high with approximately 7.5 inches of water. This maze was selected as an established and widely used device that can be used to measure associative learning and memory.

On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first (learning) trial, the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this learning trial was designated the incorrect goal during the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a transport cage to wait for the next trial. The inter-trial interval was approximately 15 (±5) seconds. Each rat was required to reach a criterion of five consecutive error-less trials to terminate the test session. The maximum number of trials in any test session was fifteen. Latency (in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial.

Animals that satisfied the above criteria within the 15-trial limit were tested for retention seven days following acquisition (animals that failed to reach criterion during acquisition were excluded from the retention phase of the experiment). The correct goal and the criterion were the same for both sessions. Dosage groups were compared for the following dependent measures:
a) Measures for acquisition included the number of trials-to-criterion, the average number of errors (incorrect turns in the maze) for each trial and the latency (in seconds) to reach the correct goal on trial 2 (a measure of short-term retention).
b) Measures for retention included the number of trials-to-criterion, the average number of errors for each trial, and the latency (in seconds) to reach the correct goal on trial 1 (a measure of long-term retention).

6) Ophthalmology: At approximately 50-60 days of age, ophthalmic exams were conducted using the males and females (a minimum of 10/sex/dose level; representing at least 20 litters per level) that were selected for perfusion at study termination. If needed to clarify the significance of findings, the animals reserved for adult brain weight measurements were also subjected to ophthalmologic examination. The exam took place in a semi-darkened room. The papillary reflex was tested using a penlight or trans-illuminator, with a mydriatic agent applied to each eye to dilate the pupil. The conjunctiva, cornea and lens were examined with a slit lamp microscope either before or after pupillary dilatation. After mydriasis, the vitreous humor, retina, choroid, and optic disc were examined using an indirect ophthalmoscope equipped with a condensing lens.

2) Postmortem Observations:

a. Maternal Animals:

Maternal animals were sacrificed by CO_2 asphyxiation on day 21 of lactation following the weaning of their respective litters. The dams were discarded without postmortem examination. Females that were sperm positive and/or had an internal vaginal plug but did not deliver were sacrificed on GD 24 without necropsy examination.

b. Offspring:

Necropsy: The offspring selected for brain weight or neuropathological evaluations were sacrificed on PND 21 or 75 (±5 days). Fl-generation animals that were found moribund (if any) while on study were sacrificed and underwent a gross necropsy examination. Tissues were collected at the discretion of the study director. In addition, randomly selected animals from Sets A-C that were used to measure fresh brain weight underwent a necropsy examination. Where required, the necropsy involved an examination of all organs (including the brain), body cavities, cut surfaces, external orifices and surfaces, with all gross abnormalities recorded. Gross lesions in neural tissues or skeletal muscle were appropriately sampled for microscopic examination. Other gross lesions were generally not collected for microscopic examination. Animals found dead (if any) underwent a necropsy examination and were disposed of without the routine collection of tissues.

Perfusion: Animals that were selected for perfusion on PND 21 (from Set D) or at study termination (from Sets A-C) were deeply anesthetized using an intraperitoneal dose of pentobarbital (approximately 50 mg/kg) and then perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by in situ fixation using universal fixative (1.0% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde) in phosphate buffer. On PND 21, only the brain (with olfactory bulbs) was collected. At study termination, the brain and spinal cord, both eyes (with optic nerves) and selected (bilateral) peripheral nerves (sciatic, tibial and

sural), the gasserian ganglion, gastrocnemius muscle, both forelimbs and physical identifier were collected. All tissues were post-fixed in 10% buffered formalin. The brain was weighed upon removal from the skull, prior to placement into formalin, and the brain:body weight ratio calculated.

Measurements: Prior to sectioning the brain for histology, a Vernier caliper was used to obtain two linear measurements (mm).
1. Anterior-to-posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; and

2. Anterior-to-posterior (AP) length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole.

These gross measurements were performed by an individual who was aware of dose group assignments.

Histology: The brain tissues from perfused animals, and any gross lesions collected at necropsy, were further processed for microscopic examination. After the gross measurements were taken, the brain was divided into eight coronal sections for microscopic examination. The eight brain sections were processed according to standard procedures for paraffin embedding, sectioned at approximately 5 µm, and examined after staining with hematoxylin and eosin (H&E). In addition, the brain sections reserved for morphometric measurements (levels 3-5 and 7) were stained using luxol fast blue/cresyl violet. Additional tissues were collected for microscopic examination from animals that were perfused at study termination. This included three levels of the spinal cord (cervical, thoracic and lumbar), the cauda equina, eyes, optic nerves and gastrocnemius muscle were embedded in paraffin and stained with H&E. Dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings and gasserian ganglia were embedded in glycol methacrylate (GMA). GMA-embedded tissues were sectioned at 2 µm - 3 µm and stained using a modified Lee's stain. Peripheral nerve tissues (sciatic, tibial and sural nerves) were embedded in GMA resin and sectioned longitudinally. The sciatic nerve was also cut in cross section.

The CHECKED (X) tissues were evaluated for adult offspring.

Central Nervous System Brain		Peripheral Nervous System Peripheral Nerves	
X	Forebrain	X	Sciatic
X	Center of cerebrum	X	Tibial
X	Midbrain	X	Sural
X	Cerebellum		
X	Pons		
X	Medulla oblongata		
Spinal Cord		Other	
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Thoracic	X	Lumbar dorsal root fibers
X	Lumbar swelling	X	Lumbar ventral root fibers
		X	Cervical dorsal root ganglion
		X	Cervical dorsal root fibers
		X	Cervical ventral root fibers
	Other		
X	Gasserian ganglion		
X	Optic nerves		
X	Cauda equina		

Micropathology and Morphometry: The tissues from high-dose animals were examined relative to those from the respective control group. If no treatment-related lesion was evident, further analysis was not performed. Any region where treatment-related neuropathology was observed underwent the following semi-quantitative analysis: Sections from all dose groups were coded and examined in randomized order without knowledge of the code. The frequency of each type of lesion was determined with the severity of each lesion graded. The code was then broken and the data evaluated for dose-effect relationships.

Selected brain regions underwent the following quantitative analysis, with the individual performing the measurements aware of dose assignments. Initially, eight linear measurements were taken. If treatment-related effects were evident following this initial evaluation, then additional measurements may have been undertaken. Two of the seven measurements involved gross measurements of the intact brain, as described above. The other five were taken from the histological sections using software calibrated with an ocular micrometer. These five measurements are described as follows:

1. **Frontal cortex** thickness (Forebrain). This measurement was of the dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm.

2. **Parietal cortex** thickness (Forebrain). This measurement was of the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm.

3. **Caudate putamen** horizontal width (Forebrain; maximum cross-sectional width). This measurement was performed on the coronal section taken at the level of the optic chiasm.

4. **Hippocampal gyrus** thickness (Midbrain). This measurement was of the full width of the hippocampal gyrus from the ventral tail of the dentate gyrus to the overlying subcortical white matter. Measurements were taken from the hippocampus from both sides of this section, and the mean value was recorded.

5. **Cerebellum height** (Cerebellum / Pons). This measurement extended from the roof of the fourth ventricle to the dorsal surface. In addition to these measurements, all brain sections from these control and high-dose male and female offspring underwent an extensive micropathological evaluation.

D. DATA ANALYSIS

1. Statistical Analyses:

The data were analyzed using the following statistical methods and significance was defined at $p \leq 0.05$ for all tests except Bartlett's test ($p \leq 0.001$):

Parameter	Statistical Method
Continuous data	Analysis of Variance (ANOVA) followed by Dunnett's test if a significant F-value was determined in the ANOVA.
Pathology data	Continuos data: Bartlett's test to analyze for homogeneity of variances among groups. Honogeneous data: ANOVA followed by Dunnett's test for pair-wise comparisons. Data with non-homogeneous variances were further analyzed using Kruskal-Wallis test followed by a Mann-Whitney U test for pairwise comparisons.
Micropathology frequency data	Chi-square test followed by a one-tailed Fisher's Exact test
Motor and locomotor activity (session data)	Repeated-measures ANOVA followed by one-way ANOVA if significant treatment x day interaction. For weeks in which there was a significant treatment effect, Dunnett's was used to determine

	significance from controls.
Motor and locomotor activity (interval data)	Two-way repeated measures ANOVA (using interval x day) followed by a Repeated-measures ANOVA to determine which weeks had a significant treatment x interval interaction. For those weeks, each interval was analyzed using a one-way ANOVA followed by Dunnett's as necessary.
Continuous FOB data	Repeated-measures ANOVA followed by one-way ANOVA and Dunnett's as necessary
Categorical FOB data	General Linear Modeling (GLM) and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts

2. Indices:

a. Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Mating Index = No. of inseminated females/No, of females co-housed with males X 100
Fertility Index = No. of pregnant females/No, of inseminated females X 100

b. Offspring viability indices: The following viability (survival) indices were calculated from lactation records of litters in the study:

Live Birth Index = No. of live pups born per litter/Total no. of pups per litter X 100
Viability Index = No. of live pups on day 4 pre-culling per litter/No, of live pups born per litter X 100
Lactation Index = No. of live pups on Day 21 per litter / No. of live pups on day 4 post-culling per litter X 100

3. Positive and Historical Control Data:

The study did not include concurrent positive controls, but references are made to previous studies to serve that purpose. Positive control data demonstrate the sensitivity of the test method to detect changes in the measured parameters. Some of these data were generated from studies using prenatal exposure while others used young-adult rats. Collectively, these studies verify the laboratory competence in evaluation of effects in neonatal animals perinatally exposed to chemicals and establish test norms for the appropriate age group. For observational measures, the data demonstrate the ability to detect major neurotoxic endpoints, including limb weakness, tremor, and autonomic signs; motor activity positive control data demonstrate the ability to detect both increases and decreases in motor activity; Pathology positive control data demonstrate the ability to detect central and peripheral nervous system pathology (separate groups were used to demonstrate each type of pathology, using acrylamide for peripheral nervous system pathology and trimethyl tin for central nervous system pathology) (Sheets, 1993). The methods were the same as those used in the study being evaluated. Statistical evaluations used to demonstrate sensitivity were the same as those used in the present study and the number of animals per test group was not greater than that used in the study under evaluation. Positive control data demonstrate inter-observer reliability for the FOB. The positive control data were collected within a reasonable time frame before the current study (e.g., the last few years). New data are collected when observational personnel or other critical laboratory elements change.

The laboratory maintains evidence of inter-observer reliability (agreement) for individuals who were involved with performing these observations (Sheets, 2004, 1993), since it is not feasible for one person to evaluate all animals on all test occasions. For measures of activity in the figure-eight maze, studies with untreated animals and rats treated with reference substances that increase (triadimefon) and decrease chlorpromazine) activity were conducted to verify the sensitivity, reliability and validity of these test procedures (Sheet,1993, 2002). Additional studies have been

performed to establish test norms for the appropriate ages under these conditions and the effects of perinatal exposure to a reference chemical (methimazole) on activity in animals tested at these ages (Sheets and Lake, 2001). The adequacy of the auditory startle test procedures has been established by performing studies with untreated animals and with rats treated with reference substances (8OHDPAT and mCPP) that alter startle response amplitude (Sheets, 2001). The adequacy of the passive avoidance test procedures has been established by performing studies with untreated animals and with rats treated with a reference substance (scopolamine) that interferes with acquisition and/or retention (Sheets, 2001; Bammer, 1982). The adequacy of the water maze test procedures was also established by performing studies with untreated animals and rats treated with a test substance (scopolamine) that interferes with acquisition and/or retention (Sheets, 2001).

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and Clinical and Functional Observations:

Moribund Animals and Animals Found Dead: No P-generation females were found dead during gestation or lactation. There were also no P-generation males found moribund or dead after initiation of the study (males were not treated).

Clinical Observations - Gestation: Compound-related clinical signs were not evident at any dose level. Findings considered incidental and unrelated to treatment included nasal stain (one control on GD 20 only), urine stain (one mid-dose animal on GD 7 only), scab formation (one control and one mid-dose animal), and hair loss (1-3 animals from each dose group) (Table 2). During gestation, such hair loss is a common finding that is associated with nest-building behavior in pregnant rats.

Clinical Observations - Lactation: Compound-related clinical signs were not evident at any dose level. Findings that are considered incidental and unrelated to treatment included a nasal stain for one mid- (days 10, 16-19) and one high- (day 17) dose female, areas of hair loss (alopecia) for one or two females from each dose group and scab formation in two controls and one mid-dose female (Table 2).

TABLE 2. Mortality and Maternal Clinical Observations [a]

Observation	Dose (mg/kg)			
	0	0.5	1.0	2.0
Gestation (Days 6-21)				
No. of females examined on GD 6	30	30	30	30
Nasal Stain	1	0	0	0
Urine Stain	0	0	1	0
Scab Formation	1	0	1	0
Hair Loss	0	1	3	1
No. of females found dead	0	0	0	0
Lactation (Days 0-21)				
No. of females examined on LD 0	29	30	29	27
Nasal Stain	0	0	1	1
Hair Loss	0	2	2	1
Scab Formation	2	0	1	0

TABLE 2. Mortality and Maternal Clinical Observations [a]				
Observation	**Dose (mg/kg)**			
	0	**0.5**	**1.0**	**2.0**
No. of females found dead	0	0	0	0

[a]Values are the number of rats with findings.
Data were obtained from Table 2 and Table 5 on page 70 and 76 of the study report, respectively.

Functional Observational Battery (FOB): Summary results of maternal FOB are presented in Table 3. All females placed on study were evaluated on days 13 and 20 of presumed gestation, and randomly selected subsets of 10 dams per dose level that were maintained on study with acceptable litters were evaluated again on lactation days 11 and 21.

Compound-related findings were not evident at any dose level. Statistically-significant findings that were considered unrelated to treatment included differences in response to handling and in ease of removal from home cage (increased incidence of vocalization for low-dose animals on GD 13 and decreased incidence for high-dose animals on GD 20, respectively), relative to controls, and a higher incidence of alopecia at the mid-dose on GD 20. Additional miscellaneous findings that were not considered to be related to treatment included red lacrimal stain and red vaginal discharge (one high-dose and one low-dose dam, respectively on GD 13), dilated pupils (one high-dose dam on GD 13 and GD 20), pupils that remain constricted (one high-dose dam on GD 13) or dilated (one high-dose dam on GD 13 and GD 20) during the pupil response test, a lesion described as a scab (one control and one mid-dose dam each on GD 20) and red nasal stain (one control dam on GD 20 and one high-dose dam on LD 11). Lastly, alopecia was seen at various dose levels at various time points. None of these findings are considered to be related to treatment, since the incidences were low, in some cases occurred in control as well as treated animals and, generally, there was no relationship to dose.

TABLE 3. Summary Data for F0-Generation Females - FOB				
Observation	**Dose Level (mg/kg/day)**			
	Control	**0.5**	**1.0**	**2.0**
Gestation Day 13				
No animals Examine	30	30	30	30
Handling-reaction to Handling		*		
Minimal resistance	29 (97)	**20 (67)**	26 (87)	28 (93)
Minima Resistance with vocalization	1 (3)	**10 (33)**	4 (13)	2 (7)
Handling-Stains (Severity)				
Not Observed (0)	30 (100)	30 (100)	30 (100)	29 (97)
Red Lacrimal (1)	0 (0)	0 (0)	0 (0)	1 (3)
Handling-Other				
Not Observed	30 (0)	29 (97)	29 (97)	30 (100)
Alopecia	0 (0)	1(3)	1 (3)	0 (0)
Handling-Other				
Not Observed	30 (100)	29 (97)	30 (100)	30 (100)
Red Vaginal Discharge	0 (0)	1 (3)	0 (0)	0 (0)
Pupil Size				
Normal	30 (100)	30 (100)	30 (100)	29 (97)
Dilated	0 (0)	0 (0)	0 (0)	1 (3)
Pupil Response-				
Dilated then Contrict	30 (100)	30 (100)	30 (100)	28 (93)
Dilated no response	0 (0)	0 (0)	0 (0)	1 (3)
Pupil are Contricted	0 (0)	0 (0)	0 (0)	1 (3)
Gestation Day 20				
No. Animals Examined	30	30	30	30
Handling-ease of Removal				*
Minimal resistance	21 (70)	23 (77)	24 (80)	**29 997)**
Minima Resistance with vocalization	9 (30)	7 (23)	6 (20)	**1 (3)**

Handling-Stains (Severity)				
Not Observed (0)	29 (97)	30 (100)	30 (100)	30 (100)
Red Nasal (1)	1 (3)	0 (0)	0 (0)	0 (0)
Handling-Other			*	
Not Observed	30 (100)	30 (100)	**27 (90)**	30 (100)
Alopecia	0 (0)	0 (0)	**3 (10)**	0 (0)
Handling-Other				
Not Observed	29 (97)	30 (100)	29 (97)	30 (100)
Scab	1 (3)	1 (3)	1 (3)	0(0)
Pupil Size				
Normal	30 (100)	30 (100)	30 (100)	29 (97)
Dilated	0 (0)	0 (0)	0 (0)	1 (3)
Pupil Response				
Dilated then Contrict	30 (100)	30 (100)	30 (100)	29 (97)
Dilated no response	0 (0)	0 (0)	0 (0)	1 (3)
Lactation Day 11				
No. Animals Examined	10	10	10	10
Handling-Stains (Severity)				
Not Observed (0)	10 (100)	10 (100)	10 (100)	9 (90)
Red Nasal (1)	0 (0)	0 (0)	0 (0)	1 (10)
Handling-Other				
Not Observed	10 (100)	8 (80)	10 (100)	9 (90)
Alopecia	0 (0)	2 (20)	0 (0)	1 (10)
Lactation Day 21				
No. Animals Examined	10	10	10	10
Handling-Other				
Not Observed	10 (100)	8 (80)	10 (100)	9 (90)
Alopecia	0 (0)	2 (20)	0 (0)	1 (10)

* significantly different from control, $p \leq 0.05$.
Severity: 0=Not observed, 1=slight, 2=Moderate to Severe.
Data were obtained from Table 15 on pages 105-128 of the study report; Number (%).

2. Body Weight and Food Consumption:

Summary results of maternal body weight and food consumption are presented in Table 4.

Gestation: The only compound-related difference in food consumption during gestation was evident at the high dose (2 mg/kg/day) during the second week of exposure. During week GD 13-20, high-dose females consumed an average 15% less feed (g/animal/day) than controls. This difference in food consumption for high-dose females was associated with an average 9% decrease in weight gain from GD 0 to 20 and a slightly lower (3%) body weight for high-dose females, compared to controls, on GD 20. There was no effect on food consumption or body weight at lower doses during gestation.

Lactation: There was no effect on food consumption or body weight during lactation at any dose level.

TABLE 4. Mean (±SD) Maternal Body Weight (g) and Food Consumption (g/animal/day)

Observation / Time	Dose Level (mg/kg/day)			
	Control	0.5	1.0	2.0
Gestation (n = 28-30)				
Mean body weight GD 0	208.6±46	212.1±2.57	209.2±2.47	210.4±2.93
Mean body weight GD 6	233.1±2.62	234.5±2.7	233.0±2.88	233.3±2.99
Mean body weight GD 13	257.0±3.33	257.8±3.34	258.9±3.07	255.9±2.89
Mean body weight GD 20	321.4±4.57	319.6±3.80	319.4±3.65	312.7±4.16
Mean weight gain GD 0-20	112.8±2.54	107.4±2.53	110.2±2.07	**102.3±2.70***
Mean food consumption GD 6-13	15.6±0.69	17.8±1.86	17.4±1.05	18.0±2.31
Mean food consumption GD 13-20	19.1±0.78	19.4±0.57	17.6±0.68	**16.2±0.74***
Lactation (n = 21-30)				

Mean body weight LD 0	246.0±3.62	249.1±3.57	248.2±3.56	241.1±3.16
Mean body weight LD 4	267.6±4.64	261.4±3.67	263.3±3.78	257.8±3.67
Mean body weight LD 7	277.4±3.95	271.3±3.17	271.8±3.79	266.2±3.08
Mean body weight LD 14	290.5±3.14	286.4±3.43	286.2±3.53	282.2±3.12
Mean body weight LD 21	283.0±3.58	281.3±3.20	279.4±3.27	276.0±3.08
Mean food consumption LD 0-7	36.3±1.78	37.3±2.82	44.7±4.48	42.1±3.47
Mean food consumption LD 7-14	49.4±1.01	47.2±0.78	48.9±0.77	46.6±0.80
Mean food consumption LD 14-21	58.6±1.23	57.4±0.95	57.3±1.14	57.1±1.10

Means for gestation period include only dams known to deliver pups (either alive or dead).
*Significantly different from control, p≤0.05
Data were obtained from Tables 3, 4, 6 and 7 on pages 72, 74, 78 and 80 of the study report, respectively.

3. Reproductive Performance:

There were no treatment-related effects on reproduction parameters at any dose level. The fertility index for high-dose females was 90.0 %, compared to 96.7 % for control and 100.0% and 96.7% for low- and mid-dose animals, respectively (Table 5). The lower value for high-dose animals is not considered a compound-related effect, as this value is well within historical control (83.3 – 100 %) [e.g., 21, 22] and there was otherwise no evidence to indicate an effect on reproduction.

TABLE 5. Summary Results of Reproductive Parameters

Observation	Dose Level (mg/kg/day)			
	Control	**0.5**	**1.0**	**2.0**
No. of Animals Co-housed [a]	30	30	30	30
No. of Animals Mated	30	30	30	30
Maternal Wastage				
No. of Dams not Pregnant	1	0	1	2
No. of Dams with Dead Pups	1	3	1	**0**
No. of Dams with one Implantaion Site	NM	NM	NM	NM
No. of Dams with Pre-Mature Delivery	0	0	0	0
Mating Index	100.0	100.0	100.0	100.0
Fertility Index (No. of Pregnant Females/No. of Insenimated Females X 100)	96.7	100.0	96.7	90.0
Gestation Length (days) [b]	21.9±0.11 [22.0] (21.0-23.0)	21.7±0.10 [22.0] (21.0-22.0)	21.9±0.11 [22.0] (21.0-23.0)	22.0±0.11 [22.0] (21.0-23.0)

[a]Number of animals assigned to each dietary level.
[b]Values are mean ± standard error, [median], and (range).
NM = Not Measured.
Data were obtained from Table1 (page 68), Table 8 (page 82) and Appendix I (page 225-232) of study report.

4. Maternal Postmortem Results (if any): Not applicable to the present study.

B. OFFSPRING (Fl GENERATION)

1. Viability and Clinical Signs:

a. Litter Data: Litter size and viability (survival) results from pups during lactation are summarized in Table 6. Litter parameters and pup viability were not affected by treatment at any dose level.

TABLE 6. Summary Results of Litter Size and Viability during Lactation				
Observation	**Dose Level (mg/kg/day)**			
	Control	**0.5**	**1.0**	**2.0**
No. of Litters	23	23	23	21
Total No. of Pups Born	269	257	274	231
Total No. of Pups Missing	1	0	1	2
Litters with Pups Missing	0	3	3	**2**
Toatl No. of Pups Found Dead	1	3	1	0
Litters with Pups Found Dead	1	3	1	0
Total No. of Pups Cannibalized	0	0	0	1
Litters with Pups Cannibalized	0	0	0	1
Mean Litter Size	11.7±0.03	11.2±0.32	11.9±0.32	11.0±0.37
No. of Stillborn Pups	0	0	0	0
Mean No. of Viable Pups				
Birth	12	11	12	11
Day 4 (Pre-cull)	12	11	12	11
Day 4 (Post-cull)	8	8	8	8
Day 21	8	8	8	8
Live Birth Index	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Viability Index	99.7±0.33	98.9±0.78	98.9±0.30	99.7±0.32
Lactation Index	100.0±0.0	98.9±0.75	100.0±0.0	98.8±0.82

Data were obtained from Table 8 on page 81 of the study report. Values are mean±standard error.

***b. Clinical Signs (Days 0-21 and Post Weaning)*:** Summary observations before weaning and post weaning are presented in Tables 7 and 8.

***Postpartum (PND 0-21)*:** There were no compound-related clinical signs during lactation in males or females at any dose level. Incidental findings that were evident on occasion in several individuals from various dose groups, including control, were limited to lacrimal stain, urine stain, various cuts (abdomen, face or ear) and bruise on the face. These findings occurred at a low incidence and did not occur in a pattern (e.g., dose-related or sustained) to indicate a relationship with exposure to the test substance (Table 7).

TABLE 7. Pups Clinical Observations – Pre-Weaning				
Observation	**Dose level (mg/kg/day)**			
	Control	**0.5**	**1.0**	**2.0**
Total No. of Pups with Observation				
Lacrimal Stain	0	0	0	1
Urine Stain	1	0	0	0
Bruise on Face	1	1	2	0
Cut on Abdomen	0	0	1	0
Cut on Face	0	0	1	0
Cut on Ear	0	0	0	1

Data obtained from pages Table 9 on page 85-86 in the study report.
Observations during lactation (days 0-21).

***Post Weaning*:** There were no compound-related clinical signs after weaning (when exposure was discontinued) in males or females at any dose level. Findings considered incidental and unrelated to treatment included urine stain (one low- and one mid-dose male), nasal stain (one mid-dose male), exophthalmia (unilateral, one high-dose male), areas of hair loss (one mid- and two high-dose males and one control female) (Table 8). Also, minor dermal lesions (scabs) were evident at various locations in individual control and treated males and/or females. These findings are not thought to be related to treatment since they also occurred after treatment had been discontinued, in some cases occurred in control animals and did not occur in a dose-related pattern.

TABLE 8. Pups Clinical Observations – Post-Weaning				
Observation	**Dose Level (mg/kg bw)**			
	Control	**0.5**	**1.0**	**2.0**
Males				
Exophthalamus				
Eye, Left	0	0	0	1 (50-71)
Hair, Alopecia				
Abdomen, Left Side	0	0	0	1 (64-71)
Limbs, Fore-Both	0	0	1 (71)	0
Thorax, Ventral	0	0	0	2 (57-71)
Lesion-Scab				
Chin	1 (50)	0	0	0
Neck, Left Side	2 (57-71)	0	0	0
Neck, Ventral	0	0	1 (50-57)	0
Shoulder, Left	2 (57,64)	2 (64,71)	4 (57-71)	0
Shoulder, Right	3 (57,64-71)	0	0	5 (57-71)
Shoulder, Both	0	1 (57-64)	5 (57-71)	1 (57-71)
Nasal Stain	0	0	2 (71)	0
Urine Stain	0	1 (71)	4 (36,71)	0
Females				
Hair, Alopecia				
Thorax, Ventral	1 (51)	0	0	0
Dead	0	1 (54)	1 (46)	0
Lesion-Scab				
Shoulder, Left	0	1 (58-65)	0	0
Shoulder, Right	0	0	1 (58-72)	0

Data obtained from Table 10 on pages 88-89 of the study report.
Observation performed during the week of PND 28,35,42,49,56,63,70
(range of days observed-observations performed once weekly).

***Animals Found Dead or Moribund (Post-Culling)*:** The number of offspring (males and females combined) found dead, moribund, or missing after culling litters on PND 4 was 0, 2, 1 and 2 for the control, low-, mid- and high-dose levels, respectively.

2. <u>Body Weight (Pre-Weaning and Post-Weaning):</u>

Pre-Weaning Body Weight: There was no difference in birth weight or PND 4 body weight in males or females at any dose level (Table 9). On PND 11, body weight was statistically decreased in high-dose males (-8%) and females (-6%). On PND 17, the difference in body weight was statistically significant in males (-8%) but not in females (-5%). On PND 21, body weight remained an average 6% less than control for high-dose males, which was not statistically significant, and recovered to within 4% less than control for high-dose females. Body weight was not affected by treatment at lower dose levels, at any time point. Low-dose females had a modestly (-6%) lower body weight (statistically different from control) on PND 11, which was not attributed to treatment since this was the only time point where body weight was considered to be different from control and there was no similar difference in females at the mid-dose nor in males at the low- or mid-dose levels.

Body weight gain was statistically decreased for various measurement intervals in high-dose animals. For PND 4-11, weight gain was statistically decreased in high-dose males (9%) and high-dose males and females combined (9%), with an 8% lower weight gain for high-dose females. Body weight gain was similarly statistically decreased on PND 4-17 in high-dose males (9%) and high-dose males and females combined (7%) and in high-dose males only on PND 11- 17 (8%). There were no statistical differences from control in low- or mid-dose males or females at any time. Low-dose females had a modestly (6%) lower, non-statistical difference from control for PND 4-11, which was not attributed to treatment since there was no similar difference in females at the mid-dose nor in males at the low- or mid-dose levels.

TABLE 9. Mean (±SD) Pre-Weaaning Pup Body Weights (g) [a]								
Postnatal Day	Dose Level (mg/kg bw)				Dose Level (mg/kg bw)			
	Control	0.5	1.0	2.0	Control	0.5	1.0	2.0
	Males				Females			
0	6.1±0.08 (23)	5.9±0.09 (23)	6.0±0.14 (23)	5.9±0.10 (21)	5.8±0.08 (23)	5.5±0.09 (23)	5.6±0.12 (23)	5.6±0.09 (21)
4[b]	10.3±0.17 (23)	9.9±0.26 (23)	10.1±0.25 (23)	9.9±0.23 (21)	9.9±0.19 (23)	9.4±0.24 (23)	9.7±0.25 (23)	9.7±0.21 (21)
4[c]	10.3±0.17 (23)	9.9±0.26 (23)	10.1±0.25 (23)	9.9±0.23 (21)	10.0±0.20 (23)	9.4±0.23 (23)	9.7±0.23 (23)	9.6±0.23 (21)
11	26.3±0.39 (23)	25.7±0.50 (23)	25.7±0.47 (23)	**24.3**±0.43 (21)**	25.6±0.44 (23)	**24.0*±0.46 (23)**	25.0±0.42 (23)	**24.0*±0.47 (21)**
17	40.6±1.58 (23)	39.0±0.67 (23)	39.4±0.62 (23)	**37.5**±0.59 (21)**	39.1±0.63 (23)	37.7±0.57 (23)	38.2±1.56 (23)	37.1±0.63 (21)
21	50.9±0.79 (23)	49.3±0.91 (23)	49.6±0.85 (23)	47.8±0.75 (21)	49.3±0.86 (23)	47.7±0.76 (23)	48.3±0.89 (23)	47.1±0.82 (21)

* Significantlly different from control, p ≤0.05. **Significantlly different from control, p≤ 0.01
[a]Data were obtained from Table 11 on pages 91-93 of the study report.
[b] Before standardization (culling). [c]After standardization (culling).

Post-Weaning Body Weight: After weaning and discontinuation of treatment, the difference in body weight for high-dose males that was evident prior to weaning persisted to study termination (an average 6-7 % less than control) (Table 10). For females, there was no difference from control after weaning at any dietary level. Body weight was also statistically less than control for the mid-dose males (5-6 %) for all but one week (PND 56) after weaning (Table 10). However, these differences from control are not attributed to treatment since they were modest, without a similar difference from control at any time prior to discontinuation of treatment, and because weights for the mid-dose males were well within the range of controls from four DNT studies conducted in 2005 - 2007 that bracket the present study.

For clarification, group average body weight data provided in the summary table and appendix are based on the mean for each litter, not for each individual pup.

TABLE 10. Mean (±SD) Post-Weaning Pup Body Weights (g) [a]								
Postnatal Day[b]	Dose Level (mg/kg bw)				Dose Level (mg/kg)			
	Control	0.5	1.0	2.0	Control	0.5	1.0	2.0
	Males				Females			
28	83.4±5.9 (23)	81.6±6.4 (23)	**79.0*±4.8 (23)**	**77.4*±5.4 (21)**	81.4±5.6 (23)	80.5±5.2 (23)	79.6±5.3 (23)	78.1±5.3 (21)
35	133.0±8.2 (23)	129±9.6 (23)	**126.1*±8.4 (23)**	**125.0*±7.3 (21)**	118.6±6.7 (23)	116.6±7.3 (23)	116.8±7.0 (23)	114.3±8.2 (21)
42	180.9±9.5 (23)	175.5±13.1 (23)	**172.3*±10.0 (23)**	**169.9*±9.2 (21)**	143.1±7.6 (23)	141.0±8.9 (23)	141.7±8.4 (23)	138.0±9.7 (21)
49	223.9±11.4 (23)	218.6±15.7 (23)	**213.7*±12.6 (23)**	**209.1*±11.8 (21)**	159.9±8.6 (23)	157.9±9.9 (23)	159.5±9.8 (23)	155.0±11.2 (21)
56	270.5±.14.7 (23)	262.7±19.0 (23)	**259.4±15.2 (23)**	**251.7±14.6 (21)**	179.7±10.0 (23)	176.2±11.6 (23)	177.0±11.3 (23)	172.1±12.0 (21)
63	305.1±15.6 (23)	294.6±21.3 (23)	**290.7*±17.8 (23)**	**285.5*±17.3 (21)**	191.4±10.5 (23)	186±12.5 (23)	188.9±12.1 (23)	183.6±12.7 (21)
70	335.2±17.6 (23)	320.5±25.7 (23)	**314.5*±22.7 (23)**	**310.2*±19.8 (21)**	201.8±11.2 (23)	196.7±10.7 (23)	197.5±12.9 (23)	193.6±13.4 (21)

*Statistical different from control, p≤ 0.05.
[a]Data obtained from Table 14 on pages 102-103 of the study report.
[b]Actual days of measurements occurred within the week of PND 28, 35, 42, 49, 56, 63, 70.

3. Developmental Landmarks:

The ages for onset of balanopreputial separation and vaginal patency were not affected by treatment at any dose level (Table 11). The age of onset for surface righting was not affected by treatment at any dose level. Although the average age of onset was lowest for control pups (average 5.2 days), there was no statistical difference at any dose level and the small differences from these concurrent controls did not increase with dose (e.g., 0.5, 0.2 and 0.3 day differences for the low-, mid- and high-dose pups, respectively) (Table 11). Pupil constriction in response to a penlight was apparent in all control and treated pups on PND 21. Therefore, there was no indication of a compound-related effect at any dose level.

TABLE 11. Mean (±SE) Age of Sexual Maturation (days) [a]

Parameter	Dose (mg/kg bw/day)			
	Control	0.5	1.0	2.0
Preputial separation	43.3±0.49	43.2±0.33	43.4±0.32	43.7±0.47
% Pups Reaching Criteria	(100)	(99)	(100)	(100)
Number of pups	23	23	23	21
Vaginal opening	31.8±0.26	32.6±0.36	32.5±0.39	31.4±0.24
% Pups Reaching Criteria	(100)	(100)	(100)	(100)
Number of pups	23	23	23	21
Surface Righting	5.2±0.19	5.7±0.18	5.4±0.20	5.5±0.18
% Pups Reaching Criteria	(100)	(95)	(100)	(98)
Number of pups	23	23	23	21

[a]Data were obtained Table 13 on from page 100 of the study report.
Values are mean ± standard error.

4. Behavioral Assessments:

a. Functional Observational Battery (FOB):

There were no compound-related findings in males or females at any dose level. Findings considered incidental and unrelated to treatment included a statistically significant increase in the average number of fecal boli during open field observations for low-dose females on PND 35, a decrease in the number of urine pools during open field observations for mid- and high-dose females on PND 60 and fewer dermal lesions (described as scabs) for low- and high-dose males on PND 60 (Table 12). Lastly, there were a few non-statistical differences from control that were also not considered to be related to treatment and included urine stain in one control male on PND 21 and one mid-dose male on PND 35, alopecia in one high-dose male on PND 60 and a dermal lesion described as a scab in two high-dose females on PND 60 (Table 12). None of these differences from control are considered to be related to treatment because the differences were minimal, were sometimes seen in controls as well as treated animals and, in some cases, were not dose related nor were they consistent across gender.

TABLE 12. Pup Functional Observational Battery Results (Incidence)

Observation	Dose (mg/kg/day)			
	Control	0.5	1.0	2.0
Males				
PND 4				
No Statistical or Treatment-Related Findings				
PND11				
No Statistical or Treatment-Related Findings				
PND 21				
Handing-Stains (Severity)				
Not Observed (0)	19 (95)	19 (100)	20 (100)	19 (100)
Yellow Urine (1)	1(5)	0 (0)	0 (0)	0 (0)
PND35				
Handing-Stains (Severity)				
Not Observed (0)	20(100)	19(100)	19(95)	19(100)
Yellow Urine (1)	0 (0)	0 (0)	1(5)	0 (0)
PND 45				

No Statistical or Treatment-Related Findings				
PND 60				
Handling-Other				
Not Observed	20(100)	19(100)	20(100)	18(95)
Alopecia	0(0)	0(0)	0(0)	1(5)
Handling-Other				
Not Observed	16(80)	**19(100) ***	19(95)	**19(100) ***
Scab	4 (20)	**0 (0)**	1(5)	**0 (0)**
Females				
PND 4				
No Statistical or Treatment-Related Findings				
PND 11				
No Statistical or Treatment-Related Findings				
PND 21				
No Statistical or Treatment-Related Findings				
PND 35				
Open Field-Defecation; No. of Boluses	0.1±0.2	**0.5±0.9***	0.1±0.4	0.1±0.2
PND 45				
No Statistical or Treatment-Related Findings				
PND 60				
Handling-Other				
Not Observed	20 (100)	20 (100)	19 (100)	17 (89)
Scab	0 (0)	0 (0)	0 (0)	2 (11)
Open Field-Defecation No. of Pools	0.9±1.7	0.3±0.6	**0.2±0.4***	**0.2±0.4***

Values represent the number of animals and % incidence (in parentheses) with or without observation.
[a]Data were obtained from Table 16 on pages 130-185 of the study report.
*Statistically different from control, $p \leq 0.05$, n= 19-20/sex/dose
Severity: = Not Observed, 1= slight, 2= Moderate to Severe

b. Motor and Locomotor Activity: Summary motor and locomotor activity data are presented in Tables 13 and 14. There were no compound-related effects on measures of motor or locomotor activity in males or females at any dose level. Moreover, there were no statistical differences from control at any dose level on any test occasion. A comparison of interval results for control and treated animals revealed no compound-related effect at any dose level. Levels of motor and locomotor activity were generally comparable to control for all test intervals on all test occasions. Moreover, there were no statistical differences from control in males or females at any dose level on any test occasion.

TABLE 13. Mean (±SD) Motor Data – Total Counts for Session [a]

Test Day	Dose (mg/kg/day)			
	Control	**0.5**	**1.0**	**2.0**
Males				
PND 13	70±65 (20)	73±51 (20)	63±54 (20)	87±105 (20)
PND 17	222±103 (20)	173±133 (20)	159±97 (20)	240±151 (20)
PND 21	315±134 (20)	312±127 (20)	297±110 (20)	324±121 (20)
PND 60	569±111 (20)	592±106 (20)	548±98 (20)	571±106 (20)
Females				
PND 13	57±52 (20)	60±54 (20)	66±53 (20)	96±93 (19)
PND 17	182±126 (20)	164±107 (20)	194±108 (20)	240±131 (19)
PND 21	334±120 (20)	292±107 (20)	299±84 (20)	350±79 (19)
PND 60	699±187 (20)	644±148 (20)	643±160 (20)	731±244 (18)

[a]Data were obtained from Table 17 on pages 187-188 of the study report.
Motor activity was not statistically different from control

TABLE 14. Mean (±SD) Locomotor Activity Data –Total Activity Counts for Sessions [a]				
Test Day	Dose (mg/kg/day)			
	Control	0.5	1.0	2.0
Males				
PND 13	6±8 (20)	5±8 (20)	6±6 (20)	12±33 (20)
PND 17	55±25 (20)	40±35 (20)	37±28 (20)	57±43 (20)
PND 21	95±36 (20)	87±29 (20)	88±31 (20)	104±38 (20)
PND 60	408±94 (20)	420±91 (20)	375±87 (20)	410±86 (20)
Females				
PND 13	4±4 (20)	8±11 (20)	8±15 (20)	8±21 (19)
PND 17	41±33 (20)	46±38 (20)	49±27 (20)	69±47 (19)
PND 21	110±37 (20)	86±32 (20)	94±28 (20)	110±30 (19)
PND 60	466±162 (20)	422±122 (20)	425±135 (20)	471±169 (18)

[a]Data were obtained from Table 18 on pages 190-191 of the study report.
Locomotor activity was not statistically different from control.

c. ***Auditory Startle Habituation:*** Startle amplitude, latency and habituation were not affected by treatment at any dose level, on any test occasion (Table 15). There were no statistical differences from control in males or females at either age.

TABLE 15. Auditory Startle Reflex Peak Amplitude Data [a]					
Test Day	Block	Dose (mg/kg/day)			
		Control	0.5	1.0	2.0
Males					
PND 23	Block 1	44±2.0	35±13	36±15	34±14
	Block 2	43±22	33±15	33±15	35±15
	Block 3	40±23	34±16	30±13	35±18
	Block 4	35±21	31±17	31±16	29±14
	Block 5	35±20	29±15	28±13	28±15
	Avg. For Total Session	**39±21**	**33±14**	**31±13**	**32±15**
	No. of Animals	18	19	18	18
	Body Weight	61	58	57	56
PND 60	Block 1	241±134	218±103	193±127	240±133
	Block 2	214±139	204±52	189±150	224±144
	Block 3	212±136	168±123	178±147	189±134
	Block 4	161±101	149±97	155±47	175±134
	Block 5	133±76	119±95	142±135	125±92
	Avg. For Total Session	**196±110**	**173±102**	**184±137**	**202±121**
	No. of Animals	20	20	19	20
	Body Weight	297	283	290	272
Females					
PND 23	Block 1	49±20	45±18	42±17	35±14
	Block 2	45±16	45±18	41±15	35±16
	Block 3	40±20	42±17	39±16	32±16
	Block 4	37±16	39±16	35±16	30±15
	Block 5	35±15	37±14	29±12	30±20
	Avg. For Total Session	**41±16**	**42±16**	**37±13**	**33±16**
	No. of Animals	19	20	20	19

	Body Weight	58	56	58	56
PND 60	Block 1	155±104	130±72	132±82	112±70
	Block 2	145±99	121±76	121±93	133±95
	Block 3	113±74	119±70	108±108	112±84
	Block 4	92±52	89±63	88±64	80±63
	Block 5	77±47	79±50	65±36	68±45
	Avg. For Total Session	**116±67**	**108±61**	**103±71**	**101±66**
	No. of Animals	20	19	20	20
	Body Weight	187	183	190	180

[a]Data were obtained from Tables 21 and 22 on pages 211-217 of the study report.
Values are (g) and mean ± SD.Values were not statistically different from control.

d. Learning and Memory Testing:

Post-Weaning - Passive Avoidance: There was no evidence of a compound-related effect on acquisition and retention in males or females at any dose level (Table 16). Moreover, there were no statistical differences from control at any dose level in either sex.

TABLE 16. Passive Avoidence Performance at PND 23 and 30 Offspring [a]

Session	Parameter	Dose (mg/kg/day)			
		Control	0.5	1.0	2.0
Males					
Session 1	Number of Animals Tested	16	16	16	16
(Learning Phase)	Number of Animals included in Analysis	16	16	16	16
	Trials to criterion	3.3±0.6	3.4±1.8	3.0±0.0	3.0±0.0
	Latency Trial 1 (sec)	30.0±23.9	56.1±46.5	47.6±33.3	40.4±21.0
	Latency Trial 2 (sec)	167.6±37.3	180.0±0.0	180.0±0.0	180.0±0.0
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Failed to Cross During Learning Phase	0 (0%)	1(6%)	0 (0%)	0 (0%)
Session 2	Number of Animals Tested	16	15	16	16
(Retention Phase)	Number of Animals included in Analysis	16	15	16	16
	Trials to criterion	2.4±0.6	2.2±0.6	2.1±0.5	2.1±0.3
	Latency Trial 1 (sec)	162.3±44.0	169.7±39.9	180.0±0.0	185.0±17.0
	Latency Trial 2 (sec)	180.0±0.0	180.0±0.0	171.9±32.5	180.0±0.0
Females					
Session 1	Number of Animals Tested	16	16	16	16
(Learning Phase)	Number of Animals included in Analysis	16	16	16	16
	Trials to criterion	3.0±0.0	3.3±1.3	3.1±0.3	3.1±0.6
	Latency Trial 1 (sec)	36.7±32.2	33.2±41.5	36.6±32.3	43.0±49.3
	Latency Trial 2 (sec)	180.0±0.0	173.3±27.0	176.6±13.5	180.0±0.0
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Failed to Cross During Learning Phase	0 (0%)	1(6%)	0 (0%)	1(6%)
Session 2	Number of Animals Tested	16	14	16	15
(Retention Phase)	Number of Animals included in Analysis	16	14	16	15
	Trials to criterion	2.5±0.7	2.6±0.9	2.3±0.6	2.3±0.6
	Latency Trial 1 (sec)	160.3±37.9	180.0±0.0	151.4±61.6	164.0±42.9
	Latency Trial 2 (sec)	171.9±22.8	166.7±31.4	175.3±18.6	172.3±30.0

[a]Data were extracted from Table 23 on pages 219-220 of the study report.
Trials to Criterion= Mean # Trials per Group ± S.D.
Latency to Trial 1 = Mean Session 1 duration (seconds) per Group ± S.D.
Latency to Trial 2 = Mean Session 2 duration (seconds) per Group ± S.D.
Failed to Meet Criterion= Number of Animals that received the shock but did not demonstrate acquisition.
Failed to Cross = Number of Animals that never received the shock.
Values were not statistically different from control.

Adult Offspring - Water Maze: Summary data for adult offspring water maze performance on PND 60 and 67 are presented in Table 17. There were no compound-related effects on acquisition or retention in males or females at any dose level. Furthermore, there were no statistical differences from control at any dose level on either test occasion.

TABLE 17. Water Maze Performance in PND 60 and 67 Adult Offspring					
Session	Parameter	Dose (mg/kg/day)			
		Control	0.5	1.0	2.0
Males					
Session 1	Number of Animals	16	16	16	16
(Learning Phase)	Trials to Criterion	6.8±2.6	7.9±2.3	9.2±3.9	6.5±1.8
	Trial 1- Errors	0.6±0.9	0.8±0.8	1.1±1.2	0.7±1.2
	Trial 1- Duration (sec)	16.6±9.3	19.4±15.3	20.1±15.0	15.6±9.7
	Trial 2- Errors	0.3±0.4	0.9±1.4	0.8±0.8	0.5±0.8
	Trial 2- Duration (sec)	10.8±7.0	18.2±6.9	18.2±15.8	15.9±11.6
	Failed to Meet Criterion	0(0%)	0(0%)	2(13%)	0(0%)
Session 2	Number of Animals	16	16	14	15
(Retention Phase)	Trials to Criterion	5.4±0.8	5.8±1.1	6.0±1.6	6.7±2.4
	Trial 1- Errors	0.6±1.0	0.3±0.4	0.4±0.6	0.4±0.8
	Trial 1- Duration (sec)	12.9±12.8	7.3±4.4	8.2±5.0	9.3±8.7
	Trial 2- Errors	0.1±0.5	0.3±0.6	0.1±0.5	0.1±0.3
	Trial 2- Duration (sec)	4.9±2.9	5.4±3.0	5.1±1.9	5.9±4.0
Females					
Session 1	Number of Animals	16	16	15	15
(Learning Phase)	Trials to Criterion	8.1±2.1	7.6±2.7	7.4±2.4	9.3±3.6
	Trial 1- Errors	1.2±0.9	0.8±1.0	1.3±1.2	0.9±1.2
	Trial 1- Duration (sec)	21.3±12.3	20.4±17.2	25.7±16.4	20.6±16.6
	Trial 2- Errors	0.6±1.1	0.7±1.2	0.5±0.6	0.9±1.2
	Trial 2- Duration (sec)	15.4±14.8	16.5±18.3	11.2±5.3	18.2±18.0
	Failed to Meet Criterion	0(0%)	0(0%)	0(0%)	2 (13%)
Session 2	Number of Animals	16	16	15	13
(Retention Phase)	Trials to Criterion	6.3±2.3	6.2±2.3	6.6±3.0	7.2±3.2
	Trial 1- Errors	0.4±0.6	0.4±0.8	0.3±0.6	0.1±0.3
	Trial 1- Duration (sec)	10.4±6.8	9.9±8.6	9.5±6.3	8.3±5.6
	Trial 2- Errors	0.2±0.5	0.3±0.7	0.1±0.3	0.5±0.9
	Trial 2- Duration (sec)	4.9±4.1	5.2±4.7	3.9±1.3	8.6±6.2

[a] Data were obtained from Table 24 on pages 222-223 of the study report
Values for rats who failed to learn during session 1 were not included in means for session 2.
Values are mean ±SD. Values were not statistically different from control.

5. Ophthalmology:

There were no compound-related lesions in males or females at any dose level. The findings in males and females from various dose groups (including control) were considered to be incidental and unrelated to treatment, due to lack of dose response, consistency by gender and/or because the incidence was within the range of historical control.

6. Postmortem Results:

a. Vendor Surveillance: There were no significant serologic titers detected in the sentinel animal/vendor surveillance evaluation.

b. Gross Pathology: There were no compound-related necropsy findings in animals that were either found dead or sacrificed on PND 21 or at study termination. Gross observations noted in perfused day 21 animals was a single eye reduced in size in a 2.0 mg/kg/day female. The only gross lesion noted in terminal perfused animals was alopecia in a 2.0 mg/kg/day female. Gross observations noted in non-perfused terminal animals was an exophthalmos in a 2.0 mg/kg/day male and crusty zones of the forelimb of one male each in control, 1.0, and 2.0 mg/kg/day groups.

c. Terminal Organ and Body Weight:

Summary results of terminal body weights and brain weights (absolute and relative) are presented in Table 18 for perfused PND 21 pups and perfused and non-perfused PND 75 terminal animals.

Body Weight: Terminal-body weights for perfused PND 21 females were statistically decreased by 12 % in the 2.0 mg/kg/day dose group. For perfused PND 21 males, terminal-body weights were not different from control at any dose level. Terminal-body weights for perfused terminal Day 75 males were statistically decreased by 14 % in the 1.0 mg/kg/day dose group, but not at the high dose. Terminal-body weights for perfused terminal PND 75 females and non-perfused PND 75 males and females were not different from control at any dose level.

Brain Weight: Absolute and relative brain weights for perfused PND 21 males and absolute brain weights in females were not affected by compound administration. The relative brain weights in the high-dose female group were statistically increased by 11 %, relative to controls, due to the significantly lower body weight for this group. Since this difference from control is due to a statistically lower body weight, this finding is not considered to be a treatment-related effect.

Absolute and relative brain weights for terminal perfused PND 75 females and terminal non-perfused PND 75 males and females were not affected by compound administration. The absolute brain weights of perfused terminal males at all doses were not affected by compound administration. The relative brain weight of mid-dose (1.0 mg/kg/day) perfused males was statistically increased, relative to controls, due to the significant decrease in terminal-body weight. This was not considered to be treatment-related, since there was no relationship with dose (i.e., no dose response). In addition, there was no effect on body weight or relative brain weight in nonperfused mid-dose males.

TABLE 18. Terminal Body Weights and Brain Weights (Absolute and Relative)

Parameter	Dose (mg/kg/day)			
	Control	0.5	1.0	2.0
Males				
PND 21 - Perfused				
Terminal Body Weight (g)	50.3±4.7 (10)	49.9±4.1 (10)	49.3±3.3 (10)	48.3±3.7 (10)
Brain, Fixed (g)	1.464±0.054 (10)	1.436±0.061 (10)	1.446±0.045 (10)	1.438±0.044 (10)
Brain, Fixed/Body Weight (%)	2.928±0.266 (10)	2.897±0.256 (10)	2.940±0.148 (10)	2.989±0.181 (10)
Terminal PND 75 (± 5) – Perfused				
Terminal Body Weight (g)	339.4±23.5 (10)	318.8±17.1 (10)	**290.8*±30.1 (10)**	317.0±19.0 (10)
Brain, Fixed (g)	1.908±0.080 (10)	1.882±0.063 (10)	1.862±0.085 (10)	1.899±0.085 (10)
Brain, Fixed/Body Weight (%)	0.564±0.032 (10)	0.592±0.039 (10)	**0.646*±0.065 (10)**	0.602±0.056 (10)
Terminal PND 75 (± 5) – Non-Perfused				
Terminal Body Weight (g)	338.9±17.9 (10)	329.1±33.2 (10)	320.7±19.0 (10)	311.9±19.9 (10)
Brain, Fixed (g)	1.919±0.058 (10)	1.953±0.035 (10)	1.959±0.055 (10)	1.908±0.064 (10)
Brain, Fixed/Body Weight (%)	0.567±0.027 (10)	0.599±0.060 (10)	0.613±0.042 (10)	0.613±0.035 (10)
Females				
PND 21 - Perfused				
Terminal Body Weight (g)	51.2±3.2 (10)	50.0±4.8 (10)	48.0±5.4 (10)	**45.2*±5.4 (10)**
Brain, Fixed (g)	1.416±0.045	1.404±0.045	1.409±0.048	1.373±0.060

	(10)	(10)	(10)	(10)
Brain, Fixed/Body Weight (%)	2.769±0.115 (10)	2.829±0.229 (10)	2.961±0.249 (10)	**3.067*±0.305 (10)**
Terminal PND 75 (± 5) – Perfused				
Terminal Body Weight (g)	205.3±24.4 (10)	207.9±16.6 (10)	203.0±20.7 (10)	198.9±14.1 (10)
Brain, Fixed (g)	1.754±0.089 (10)	1.751±0.056 (10)	1.729±0.048 (10)	1.736±0.074 (10)
Brain, Fixed/Body Weight (%)	0.864±0.097 (10)	0.846±0.066 (10)	0.858±0.072 (10)	0.876±0.055 (10)
Terminal PND 75 (± 5) – Non-Perfused				
Terminal Body Weight (g)	207.0±12.8 (10)	203.7±14.9 (10)	201.9±15.3 (10)	200.9±16.8 (10)
Brain, Fixed (g)	1.802±0.046 (10)	1.786±0.046 (10)	1.812±0.055 (10)	1.744±0.100 (10)
Brain, Fixed/Body Weight (%)	0.874±0.060 (10)	0.881±0.070 (10)	0.902±0.066 (10)	0.873±0.080 (10)

[a]Data were obtained from Table pages Table OW1K-SUM on pages 942-947 of the study report
* Statistically different from control, $p \leq 0.05$.

***d. <u>Brain Morphometry and Micropathology Measurements</u>*:** As shown in Table 19, there were no treatment-related findings in gross and micropathology brain measurements on PND 21 or terminal animals. Also, there were treatment-related microscopic findings in brain tissues from perfused terminal high-dose (2 mg/kg/day) males and females.

TABLE 19. Gross Necropsy and Micropathology Brain Measurements

Parameter	Dose (mg/kg/day)			
	Control	**0.5**	**1.0**	**2.0**
Males				
<u>Gross Measurements</u>				
PND 21				
Ant/Post Cerebrum Length (mm)	13.80±31 (10)	13.83±0.35 (10)	13.95±0.22 (10)	13.74±0.25 (10)
Ant/post Cerebellum (mm)	7.29±0.22 (10)	7.07±0.36 (10)	7.02±0.25 (10)	7.02±0.30 (10)
Terminal PND 75 - Perfused				
Ant/Post Cerebrum Length (mm)	15.00±0.33 (10)	14.66±0.47 (10)	14.80±0.34 (10)	14.89±0.46 (10)
Ant/post Cerebellum (mm)	7.81±0.19 (10)	7.78±0.41 (10)	7.70±0.47 (10)	7.71±0.50 (10)
<u>Microscopic measurements</u>				
PND 21				
Frontal Cortex (mm)	1.813±0.010	--	--	1.827±0.010
Parietal Cortex (mm)	1.918±0.007	--	--	1.943±0.004
Caudate Putamen (mm)	3.73±0.029	--	--	3.103±0.011
Hippocampal Gyrus (mm)	1.663±0.005	--	--	1.683±0.014
Cerebellum (mm)	4.823±0.055	--	--	4.779±0.066
Terminal PND 75 - Perfused				
Frontal Cortex (mm)	1.671±0.012	--	--	1.728±0.015
Parietal Cortex (mm)	1.829±0.005	--	--	1.859±0.008
Caudate Putamen (mm)	3.275±0.019	--	--	3.236±0.026
Hippocampal Gyrus (mm)	1.794±0.016	--	--	1.779±0.024
Cerebellum (mm)	4.695±0.207	--	--	4.747±0.150
Females				
<u>Gross Measurements</u>				
PND 21				
Ant/Post Cerebrum Length (mm)	13.67±0.36 (10)	13.62±0.26 (10)	13.62±0.29 (10)	13.44±0.25 (10)
Ant/post Cerebellum (mm)	7.03±0.24 (10)	7.06±0.38 (10)	7.08±0.38 (10)	7.21±0.28 (10)
Terminal PND 75 - Perfused				
Ant/Post Cerebrum Length (mm)	14.34±0.27 (10)	14.31±0.35 (10)	14.46±0.21 (10)	14.35±0.28 (10)
Ant/post Cerebellum (mm)	7.69±0.34 (10)	7.54±0.21 (10)	7.55±0.44 (10)	7.74±0.28 (10)
<u>Microscopic measurements</u>				
PND 21				

Frontal Cortex (mm)	1.770±0.014	--	--	1.835±0.011
Parietal Cortex (mm)	1.877±0.014	--	--	1.848±0.011
Caudate Putamen (mm)	3.070±0.013	--	--	3.015±0.023
Hippocampal Gyrus (mm)	1.649±0.018	--	--	1.671±0.008
Cerebellum (mm)				
Terminal PND 75 - Perfused				
Frontal Cortex (mm)	1.649±0.002	--	--	1.692±0.012
Parietal Cortex (mm)	1.796±0.004	--	--	1.804±0.009
Caudate Putamen (mm)	3.271±0.022	--	--	3.218±0.017
Hippocampal Gyrus (mm)	1.723±0.024	--	--	1.676±0.049
Cerebellum (mm)	4.574±0.133	--	--	4.676±0.081

[a]Data were obtained from Table pages 942-943, 944-945 and 948-951 of the study report
Values are mean ± SD. -- = not evaluated.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

In summary, the following observations were noted.

The analytically-confirmed doses administered to the dams during gestation and lactation were 0, 0.51 0.97 and 1.90 mg/kg/day. There was no effect on reproduction parameters at any dose level.

Maternal - Compound-related effects consisted of the following:
0.51 mg/kg/day - There were no treatment-related findings during gestation or lactation.
0.97 mg/kg/day - There were no treatment-related findings during gestation or lactation.
1.90 mg/kg/day - Compound-related effects consisted of decreased food consumption during GD 13-20, with an associated decrease in body weight gain from GD 0-20. Body weight was slightly reduced, relative to controls, on GD 20.

Offspring - Compound-related effects were limited to the following:
0.51 mg/kg/day - There were no treatment-related findings.
0.97 mg/kg/day - There were no treatment-related findings.
1.90 mg/kg/day - Body weight was statistically and/or non-statistically decreased in males and females during lactation. These differences from control were statistically significant in males on PND 11 and 17 (- 8%) and in females on PND 11 (-6%) and were associated with significant reduced body weight gain that began on PND 4-11. In addition, body weight was non-statistically decreased on PND 21 in males. This difference in body weight for males persisted to study termination (statistically decreased 6-7% compared to control).

Thus, the LOAEL is 2.0 mg/kg bw/day for maternal animals and offspring, based on decreased body weight and body weight gain, and decreased food consumption (dams only).

Conclusion:
Based on these collective results, it is concluded that technical grade flumethrin, administered at the highest dose of 2.0 mg/kg/day to pregnant rats from GD 6 through LD 21, is not a developmental neurotoxicant. The only compound-related effects occurred at the highest dose level and consisted of decreases in body weight and body weight gain, and in food consumption (dams only).

Tabular Summary

Dosages (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Compound-Related Effects at LOEL
Maternal Animals 0, 0.51, 0.97 and 1.90	1.0	2.0	↓ Body weight, body weight gain and food consumption during Gestation
Offspring			↓ Body weight and body weight gain

0, 0.51, 0.97 and 1.90	1.0	2.0	

A. REVIEWER COMMENTS: This study in rats is classified as **Acceptable/Guideline** and satisfies the guideline requirements for (OPPTS 870.6300) for a developmental neurotoxicity.

C. PROTOCOL DEVIATIONS

1. The US-EPA OPPTS 870.6300 study design used in this study was revised to address the following additional requirements that were outlined in a 1999 Data Call-In (DCI) Notice issued by the U.S. EPA for several organophosphorus insecticides:
    - Increase the number of pups for neuropathological evaluation on postnatal day (PND) 11 from 6/sex/dose level to 10/sex/level.
    - Extend the period of exposure to include the period from day 6 of gestation through PND21, rather than discontinuing exposure on PND11.

    These requirements were met by:
    (1) Extending exposure by lactation to postnatal day 21 (rather than day 11) and evaluating brains from 21-day-old offspring (rather than 11-day-old animals) for morphometry and micropathology, and
    (2) Using a sample size of 10 (rather than 6 animals) per sex per dose level.

    All changes in or revisions to the approved protocol were implemented by written amendment, signed and dated by the study director and appropriate management and maintained with the protocol.

2. The following four protocol deviations that occurred during the study were reported. They did not significantly impact the outcome of the study.
    - On 7/17/06, the high temperature in animal room 301 was recorded as 80ºF and the animal room lights turned off momentarily. These deviations occurred because the electrical substation Electrical Lighting and Electrical Power Panel Breakers tripped on ground fault. These deveiations did not impact the outcome of the study. (Reported on page 16)
    - Animal EVO104 was inadvertently dosed on GD 24 (6/8/06). Since this animal was not pregnant and was sacrificed on GD 24, this deviation did not adversely affect the outcome of the study. (Reported on page 21).
    - On 8/2/06, one reading of the Y axis for L in chamber two was 121.1 dB. Since the other three L measurements taken for chamber two were all within the acceptable range, this deviation had no adverse impact on the outcome of the study. (Report on page 26).
    - During the week of 7/17/06 through 7/23/06, animal study room 301 was inadvertently not disinfected. This deviation did not adversely affect the outcome of the study. (Reported on page 35).

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